Michigan Department of Community Health
MDCH
Jennifer M. Granholm, Governor
Janet Olszewski, Director

LabLink

Michigan Department of Community Health
Bureau of Laboratories

"Quality Laboratory Science for Healthier People and Communities"

Vol. 11 No. 3 **Summer 2006**

The New Face of *Clostridium difficile*

Patricia Somsel, Dr.P.H.
Division of Infectious Diseases

In the 1970s, antibiotic-associated diarrhea, a complication of lincomycin and clindamycin use, was recognized as a "new disease." In the past 30 years, this condition has been associated with many antibiotics, as well as some chemotherapy agents that also have antibacterial activity. The spectrum of disease extends from simple diarrhea (*Clostridium difficile*-associated diarrhea or CDAD) to pseudomembranous colitis, sepsis and death. *C. difficile* is part of the normal flora in up to 3% of healthy adults but may cause disease in the presence of antibiotics that disrupt normal bowel flora. Disease causing strains of *C. difficile* produce one or both of two exotoxins, Toxin A and Toxin B. Toxin A, an enterotoxin, stimulates fluid secretion in the large bowel. Toxin B, a potent cytotoxin, causes death of cells of the colonic mucosa, resulting in mucosal injury, inflammation and development of characteristic pseudomembranes. Strains, which lack the genes for toxin production, produce no disease.

Outbreaks of disease have been recognized in medical facilities, especially in nursing homes. Persistence of spores and resultant recurrences greatly complicates control of this disease.

Since 2000, the epidemiology of *C. difficile* has evolved into more severe disease and a higher death rate among those with CDAD. From 2000 to 2003, there was more than a doubling in the listing of CDAD as the first diagnosis in hospital discharges, from 25,000 to 54,000. Likewise, the number of discharges for which CDAD was listed as any diagnosis nearly doubled from 98,000 in 1996 to 178,000 in 2003. A significant linear trend in increased rates was found only for the Midwest, although rates appear to be increased in all regions from 2000 to 2003. The rates in persons 65 and older was several fold higher than that of persons ages 45-65, which was in turn higher than that of persons under 44 years of age.

Characterization of the recent epidemic isolates indicated one related strain with a greater level of production of exotoxins and an additional binary toxin. While the function of the latter is not known, it is carried by 100% of isolates of the recent epidemic strain but only 6% of previous isolates in the U.S., Canadian and European hospitals circulating prior to 2000. This strain also may carry an 18-bp deletion that could act in a feedback loop to down-regulate toxin production in non-epidemic strains. This could explain the high levels of toxin production seen in the epidemic

strains, up to 16 times more toxin A and 23 times more toxin B than non-epidemic strains.

Risk factors identified previously include older age, exposure to health care facilities, either acute or long-term, and the recent use of antibiotics. Recent studies suggest decreased host defenses in older individuals, including decreased stomach acid due to achlorhydria or increased use of histamine-2 receptor blockers or proton-pump inhibitors, may play a role.

Now *C. difficile* is being diagnosed in patients who lack the normal risk factors previously associated with disease. Community-acquired-*C. difficile* cases (CA-CDAD) may be difficult for clinicians to recognize because the patients are younger, often have not been in health care facilities until their illness, and had little or no antibiotic exposure. An article published in *Morbidity and Mortality Weekly Report* reviewed cases of *C. difficile* from four states in 2005, unusual for occurrence in otherwise healthy individuals with minimal to no exposure to health care settings. The cases included a 31 year-old woman, 14 weeks pregnant, who died following 21 days of hospitalization, despite seemingly appropriate antibiotic therapy and subtotal colectomy. She tested positive for *C. difficile* upon admission for intermittent diarrhea of three (3) weeks duration. She had received trimethoprim-sulfamethoxazole for a urinary tract infection three (3) months previously. Another case involved a 10 year-old girl hospitalized for intractable diarrhea of two (2) weeks duration, with projectile vomiting and abdominal pain. She was found to be positive for *C. difficile* upon admission, despite not having received antibiotics in the previous year. In total, ten peripartum and 23 CA-CDAD cases were reviewed. Eight (24%) of the patients reported no antibiotic use in the three (3) months before their illness. The symptoms of patients are also notable for the presence of high fever and bloody diarrhea, symptoms that might lead a community physician to seek alternative diagnoses such as *E. coli* O157:H7, to dispense inappropriate antibiotic administration and delay appropriate therapy. Like CA-*Staphylococcus aureus* infections, recognition of CA-*C. difficile* disease may require far greater awareness of the agent than currently found in the medical community.

References:

Centers for Disease Control and Prevention, "Severe *Clostridium difficile*-Associated Disease in Populations Previously at Low Risk – Four States," 2005, *MMWR Morb Mortal Wkly Rep*, 2005;54:1201-5.

McDonald, LC, M Owings, DB Jernigan, "*Clostridium difficile* Infection in Patients Discharged from US Short-stay Hospitals 1996-2003," *Emerg Infect Dis* [serial on the Internet], 2006 Mar.

McDonald, LC, GE Kilgore, A Thompson, et. al., "Emergence of an Epidemic, Toxin Gene Variant Strain *Clostridium difficile* Responsible for Outbreaks in the United States between 2000 and 2004'" *N Eng J Med*. 2005;353:2433-41.

Bureau of Laboratories Vision

The Bureau of Laboratories is a stronger, more diverse team within an integrated public health system. We utilize advanced technology and innovative leadership to provide comprehensive public health services in our dynamic global community.

Bureau of Laboratories Mission

We are dedicated to continuing leadership in providing quality laboratory science for healthier people and communities through partnerships, communication and technical innovation.

Michigan Experiencing Surge in Terrestrial Rabies Cases

Kimberly Signs, D.V.M.
Bureau of Epidemiology

The Michigan Department of Community Health's Bureau of Laboratories has detected 23 cases of rabies out of 986 (2.3%) animals tested through June 26, 2006. These include 18 bats, four horses, and one skunk. Over the same time period in 2005, nine positive animals had been detected out of 965 (0.9%) submissions. Typically, July and August are the busiest months in the MDCH laboratory for rabies testing. This year is unusual not only in the number of positive animals detected in the first half of the year, but also four horses have been found to be positive for rabies; the most in a single year since 1999.

Both 2005 and 2006 animal rabies statistics reflect higher case numbers due to the skunk-strain of rabies. While bat rabies is detected sporadically throughout the state, the skunk-strain of rabies has, in recent years, only been detected in southeast Michigan and "the thumb" counties. In 2005, a total of 13 animals, including seven skunks, four cats, a sheep and fox were infected with this strain of rabies. For 2006, to date, four horses and one skunk have been detected with this strain. Diseases in wildlife often experience natural cycles of high and low incidence and rabies is no exception. Rabies of terrestrial animals, such as skunks, are more likely to spill over into other unvaccinated domestic animals such as cats and horses as they are more likely to encounter a sick skunk. Bat rabies rarely spills over into other species.

For most domestic species of animals including dogs, cats, ferrets, horses, cattle, and sheep, there are licensed rabies biologics available. Only dogs and ferrets are required by law to have rabies vaccinations in Michigan, but other species whose daily activities could expose them to potential rabies-infected animals should be vaccinated. For up-to-date information on rabies surveillance data please visit the Emerging Diseases website at www.michigan.gov/emergingdiseases. Click on the "Rabies" topic.

Congratulations to Dr. Jeff Massey (MDCH Houghton Laboratory) on his latest publication!

For the article "Molecular Epidemiology and Cluster Analysis of Human Listeriosis in Three U.S. States," see *The Journal of Food Protection*, Vol 69, No. 7, 2006, pages 1680-1689

Expanded Newborn Screening

Harry C. Hawkins, B.S.
Newborn Screening Section

The MDCH newborn screening laboratory has completed a one-year pilot of tandem mass spectrometry testing to detect 38 additional disorders. The new disorders are on a list commissioned by the Health Resources and Services Administration (HRSA) and developed by the American College of Medical Genetics in 2005. The paper, entitled Newborn Screening: Toward a Uniform Screening Panel and System, can be found at http://mchb.hrsa.gov/screening/ .

The list of disorders recommended for screening was the result of a comprehensive effort that reviewed 84 conditions. These disorders were ranked by clinicians, geneticists and other experts according to clinical characteristics, diagnosis, follow-up, treatment, management of the condition, and analytical characteristics of the test. The highest-ranking disorders were assigned to one of three groups; a core panel (screened directly), secondary targets (available through multiplex testing), or inappropriate for screening.

The core panel recommended by the March of Dimes and HRSA consists of the 11 disorders previously reported in Michigan, an additional 17 disorders detected with tandem mass spectrometry, cystic fibrosis screen, and newborn hearing screen. There are 25 secondary target disorders on the list; 21 of which were included in the pilot evaluation. In May 2006, following a major upgrade to the laboratory information system, MDCH began providing a comprehensive report of the all disorders from tandem mass spectrometry.

The currently reported disorders (49) were condensed to six groups to fit on one report page. The patient's quantitative result and expected values are listed only when abnormal findings are observed; otherwise, patient results are reported as "Within Normal Limits" for that disorder/ analyte group. The disorder/analyte(s) groupings are amino acid disorders, fatty acid oxidation disorders, organic acid disorders, endocrine disorders, enzyme disorders, and hemoglobinopathies.

The complete list of disorders currently screened for at MDCH may be reviewed at http://www.michigan.gov/mdch/0,1607,7-132-2942_4911_4916-64851--,00.html . For any questions, please contact Harry Hawkins at HawkinsH@michigan.gov or at 517-335-8095.

Laboratory Preparedness- Chemical Exposure Event

Ninah M. Sasy, MT
Division of Chemistry and Toxicology

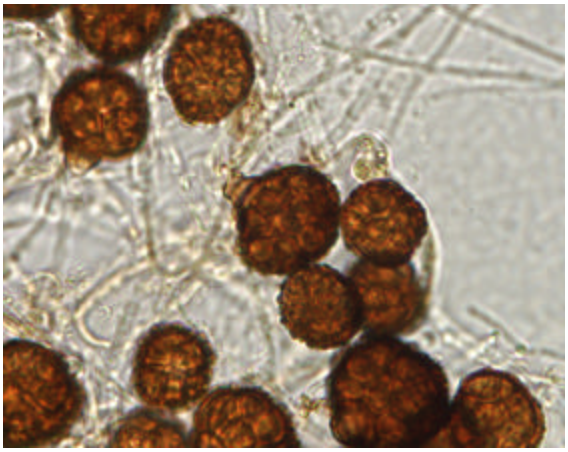
In an effort to enhance local preparedness for a chemical exposure event, MDCH, Bureau of Laboratories has assembled a Chemical Terrorism (CT) Packaging and Shipping Kit. This kit will be distributed to hospital and regional laboratories across Michigan. The CT Packaging and Shipping Kit contains the material necessary to properly transport approximately 56 blood specimens or 20 urine specimens collected from victims of a chemical event. This kit should be stored in the laboratory as part of facility and regional emergency preparedness plans. If a large-scale event occurs, having kits in each region may facilitate a more timely response.

There are unique requirements for collecting specimens from victims of a chemical exposure event. MDCH continues to offer training for hospital staff in the proper collection and handling of these specimens. This training, "Chemical Terrorism: What the Hospital Should Know," can be presented, at no charge, at your hospital by the Chemical Terrorism Laboratory Coordinator. Beginning this fall, the training will also be available online, also at no charge. To schedule an on-site training session, please contact Ninah Sasy at 517-335-9152 or sasyn@michigan.gov .

FUN FUNGI.....
Epicoccum species

Sandy Arduin MT(ASCP) & Bruce Palma MT(ASCP) - Mycobacteriology/Mycology Unit

The genus Epicoccum contains a single species, *Epicoccum pupurascens.* It is commonly isolated from air, soil, foodstuff, and some animals and textiles. *E. pupurascens* is a common causative agent of leaf spot in various plants. There are no documented cases of infection in humans or animals. It is occasionally isolated from clinical samples but is considered a contaminant in these instances. Colonies are felty to wooly and grow rapidly. Colony color is yellow, orange, red or brown on the surface with a deep brown reverse. *E. pupurascens* often produces a diffusible pigment, which turns the inoculated media yellow, orange, red or brown. Black dots may appear on the surface of the colony. These are clumps of conidia. Conidia are formed on short conidiophores that are little differentiated from the hyphae and often form sporodochia (compact clusters). The conidia are brown, round, muriform (multiple transverse and vertical septa), and have a funnel shaped base.

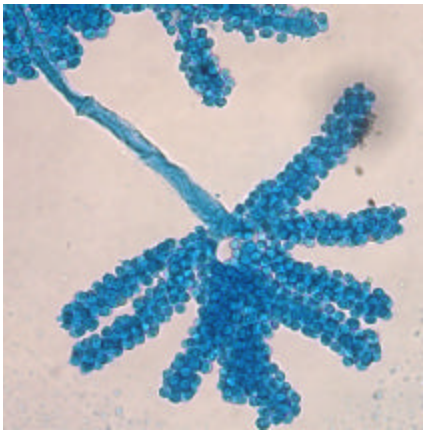


References:

1. St-Germain, Guy, Summerbell, Richard. 1996. *Identifying Filamentous Fungi, A Clinical Laboratory Handbook.* Star Publishing Co. Belmont, CA.

2. www.doctorfungus.org/thefungi/epicoccum.htm

Last Issue's Picture Quiz Answer:



Chromelosporium* anamorph of *Peziza

Chromelosporium species are fungi commonly found in peaty soil, steam-sterilized soil, on the walls of greenhouses and in mushroom beds. They are less commonly found in forest soils. Macroscopically, colonies are velvety to tufted and are white, rose, ochraceous or brown. Microscopically, conidiophores are stout, erect, hyaline to ochraceous and are dichotomously branched (branching into two more or less equal branches) near the apex. These club-like branches are covered with globose conidia on short denticles.

This Issue's Picture Quiz: What Mould is this?

Infectious Disease Case Study in Michigan Part 3

William G. Crafts, MT (ASCP) and
Kristine Smith, MT (ASCP)
Bacterial and Parasitic Serology Unit

Case Summary

The following case study (see *LabLink* articles Vol. 11 No. 1 and 2, 2006) documents the first reported case of *Borrelia burgdorferi* [(Lyme Borreliosis – (LB)] and *Anaplasma (Ehrlichia) phagocytophila* (Anaplasmosis) coinfection in the state of Michigan. The patient resided in an area endemic for Lyme disease and stated she removed two attached engorged ticks a few weeks prior to the onset of myalgia, nausea, headache, abdominal pain and tenderness. Laboratory testing (see Table 1) revealed leukopenia, thrombocytopenia and elevated hepatic transaminases (ALT and AST). The presence of Lyme IgM and IgG antibodies could have been due to her previous infection in 2002, although IgM antibodies are expected to wane after three years. Acute and convalescent sera Lyme EIA index values were 4.95 and 6.29 respectively and all three significant IgM WB bands were present. In addition, leukopenia, thrombocytopenia and elevated liver enzymes, which are not usually seen with latent LB, all indicate a recent exposure. *A. phagocytophila* IgG antibody titers were 1:1024 (normal range < 1:64). Clinical symptoms, patient exposure to an area endemic for Lyme disease, confirmation of tick bite, and laboratory testing confirmed a recent coinfection with *B. burgdorferi* and *A. phagocytophila*.

Table 1 Laboratory Testing Results

Test	Collected 6/13/05	Collected 6/20/05	Collected 7/7/05
WBC count K/mm^3	2.6 L	6.4	Not done
Platelet count K/mm^3	119 L	229	Not done
ALT (SGPT) U/L	89 H	79 H	Not done
AST (SGOT) U/L	81 H	66 H	Not done
Lyme IgG/IgM EIA Index	Not done	4.9	6.3
Lyme IgM WB Bands observed	Not done	Pos 23, 30, 34, 41, 58, 66, 93	Pos 23, 30, 41, 58, 66, 93
Lyme IgG WB Bands observed	Not done	Pos 18, 23, 30, 37, 39, 41, 45, 66, 93	Pos 18, 23, 30, 37, 39, 41, 45, 66
Ehrlichia chaffeensis IgG IFA titer	Not done	< 1:32	< 1:32
Anaplasma (Ehrlichia) *phagocytophila* IgG IFA titer	Not done	= 1:1024	= 1:1024
Rickettsia prowazekii IgG IFA titer	Not done	< 1:32	< 1:32
Rickettsia typhi IgG IFA titer	Not done	< 1:32	< 1:32

L= low value
H= high value

Lyme Borreliosis

Background

Lyme disease, now referred to as Lyme Borreliosis (LB), is the most common vector-borne disease in North America and represents a major public health concern. Since 1982, more than 200,000 LB cases in the United States (U.S.) have been reported to the Centers for Disease Control and Prevention (CDC) with a national incidence rate of 8.2 cases per 100,000. In 1990, LB became a nationally notifiable disease, and the current CDC case definition for surveillance purposes includes either physician-diagnosed EM lesions = 5 cm in diameter or at least one latent symptom (arthritis, neurologic, or cardiac) along with reactive Lyme serology.

Etiologic Agent, Transmission and Pathogenesis

LB in the U. S. is caused by infection with the spirochete bacterium *Borrelia burgdorferi* and is transmitted by ticks of the *Ixodes ricinis* (*persulcatus*) complex. This complex includes *Ixodes pacificus*, predominately located in the northwestern U.S., and *Ixodes scapularis,* typically found in the northeast and Midwest regions of the U.S. Nymphal stage ticks and some adult ticks feed in the late spring and early summer and are responsible for transmitting the organism to animals and humans. Female ticks, due to their long feeding periods and large blood meals, are primary vectors of LB, however the debate continues whether infected male ticks transmit disease.

Since *B. burgdorferi* resides in the midgut of infected ticks, it must be attached to its host for approximately 48 hours for effective transmission. *B. burgdorferi* alters its outer membrane proteins depending upon the environment in which it resides. The OspA protein, which is the predominant outer surface membrane protein, is expressed in the tick vector while OspC is expressed while in the mammalian host. During infection, the organism differentially expresses various surface membrane proteins that enable it to evade or impede immunologic destruction.

Clinical Symptoms

Lyme disease is a complex, multisystem, multistaged illness and is classified into acute, early dissemination and chronic stages. In its acute stage, approximately 40% of patients exhibit a localized skin infection (bull's eye lesion, erythema migrans [EM] rash) that appears within 7-14 days of exposure. Early dissemination can occur within days, weeks or months and represents hematogenous spread of the spirochete resulting in multiple secondary EM lesions, meningitis, radiculoneuritis, myocarditis and/or arthritis. Fever, malaise, headache, fatigue and myalgia often accompany early infection. Chronic stage occurs months or years following infection and can be associated with acrodermatitis chronic atrophicans, chronic arthritis and encephalopathies. Arthritis, often in the knee, is the most common late manifestation of LB in North America. During the early stage, patients can be successfully treated with oral antibiotics (e.g., doxycycline), but if left untreated, may develop systemic complications requiring extensive antimicrobial therapy.

Coinfections

In addition to *B. burgdorferi* and *A. phagocytophila*, ticks harbor and transmit other bacterial, protozoan and viral pathogens. *Ehrlichia chaffeensis, Babesia microti, Rickettsia* spp. and a variety of viral pathogens may exist alone or in any combination in ticks.

In endemic areas of the U. S., recent studies have shown that 50% of the *Ixodes* tick population carries either human granulocytic ehrlichiosis (HGE) or *B. microti*. The frequency of coinfection with *Borrelia* and *B. microti* is estimated to be 8-11%, while *Borrelia* and HGE is closer to 4%. In a similar study of 96 Wisconsin LB patients, immunoserology showed coinfection with *B. microti* and *A.*

phagoctyophila in 9.4% (2). These coinfections may be difficult to diagnose due to similarity of symptoms, possible serologic cross-reactions and difficulty in testing. Since treatment may differ for each pathogen involved, correct diagnosis is crucial.

Serologic Testing

MDCH follows a two-step protocol currently recommended by the CDC. Serum specimens are screened using a *B. burgdorferi* B31 strain whole-cell sonicated, enzyme immunoassay (EIA). IgG and/or IgM western immunoblot assay (WB) is then performed on all specimens demonstrating a positive or equivocal EIA result. Two out of three IgM bands constitute a positive IgM result, and five out of ten IgG bands constitute a positive IgG result (see Table 2). The decision to perform IgM WB, IgG WB or both is based on clinical symptoms, date of onset and travel history provided by the physician. This information must be documented on the MDCH virology test requisition form (DCH-0583). Due to the low sensitivity during acute disease, serologic testing is not recommended for patients with a current EM rash.

Although the two-step protocol is less sensitive during acute LB, it is more specific and leads to fewer false positive IgM WB results. False positive immunoblots are usually due to low-level reactivity to the 41 kDa and 23 kDa antigens that may be found in the normal population.

Problems Associated with the Serologic Testing

The diagnosis of LB is based on clinical signs/symptoms, travel history to endemic locations, possible tick exposure, and serologic testing. Laboratories not adhering to the two-step testing protocol or physicians requesting WB as a screening test may result in the over-diagnosis of LB. Results must be interpreted with caution in those patients exhibiting IgM positive WB, IgG negative WB and reactive EIA, in the absence of acute disease and typical exposure history. Paired sera analysis using the two-step testing protocol enhances diagnostic accuracy in this situation.

Table 2 Breakdown of *Borrelia* Protein Band Definitions

Band	Band Definition
*18 kDa	P18 flagellin fragment
#*23 kDa	OspC - specific for *B. burgdorferi*
*28 kDa	OspD - specific for *B. burgdorferi*
*30 kDa	OspA - substrate binding protein
31kDa	OspA - will be present in vaccinated individuals
^34 kDa	OspB - specific for *B. burgdorferi*
^37 kDa	FlaA gene product - specific for *B. burgdorferi*
#*39 kDa	BmpA - specific for *B. burgdorferi*
#*41 kDa	FlaB – most common band found in non-infected individuals
*45 kDa	Unknown
*58 kDa	Unknown
*66 kDa	Oms66
*93 kDa	Immunodominant cylinder antigen associated with flagellum - specific for *B. burgdorferi*

*IgG bands used in CDC interpretation
#IgM bands used in CDC interpretation
^ Not included in CDC interpretation
Osp – outer surface protein
Oms – outer membrane spanning
Bmp – bacterial membrane protein
Fla - flagellin

Retrospective analysis of true LB cases over the past 7 years at MDCH shows a high correlation between EIA index values = 2.0 and positive WB results. True LB cases were defined as patients exhibiting EM rash, supportive clinical signs/symptoms, travel history to endemic locations, evidence of tick bite, and laboratory confirmation.

The use of different strains of *B. burgdorferi* in immunoblot testing and variations in test result interpretation add to the confusion surrounding the serologic diagnosis of LB.

Culture

B. burgdorferi spirochetes are fastidious, microaerophilic bacteria that grow best at 33-35°C in Barbour-Stoenner-Kelly (BSK) medium. The media, inoculated with skin biopsies or saline washes of EM lesions, blood, cerebrospinal fluid (CSF) or tick-midgut material, is microscopically examined over a 90-day period for the presence of motile, loosely coiled spirochetes. A positive culture, confirmed using monoclonal DFA conjugate (specific for *B. burgdorferi*), provides a definitive diagnosis. The recovery of live organisms is highest when the BSK media is inoculated immediately following specimen collection. Because culture requires a phase-contrast microscope and experienced microbiologists, few labs offer this service. Borrelia culture is available at MDCH.

PCR

Although PCR is more sensitive than culture, especially in joint fluid detection, it has not been standardized for the routine diagnosis of Lyme disease. PCR is also more sensitive than serologic testing. PCR is not currently available at MDCH.

Treatment

Lyme disease and its various manifestations are typically treated with 14-21 days of oral antibiotics. Neuroborreliosis may require intravenous therapy for a longer duration. For early disease and early late disease with dissemination, doxycycline for 14-21 days is recommended for patients 8 years old and above. This regimen is also effective against Ehrlichia/Anaplasma infection. Amoxicillin is often prescribed for children younger than 8 years old and pregnant women.

References:

1. Aguero-Rosenfeld, M. E., Wang, G., Schwartz, I. and Wormser, G. P. 2005. Diagnosis of Lyme Borreliosis. *Clin. Microbiol. Reviews* , 18.3:484-509.

2. Mitchell, P. D., Reed, K. E. and Hofkes, J. M. 1996. Immunoserologic evidence of coinfection with *Borrelia burgdorferi*, *Babesia microti*, and human granulocytic *Ehrlichia* species in residents of Wisconsin and Minnesota. *J. Clin. Microbiol*. 34:724-727. (Abstract).

3. Goodman, J. E., Dennis, D. T. and Sonenshine, D. E. (eds.) 2005. Lyme Borreliosis IN: Tick-Borne Diseases of Humans. pg. 176-206.

4. Goodman, J. E., Dennis, D. T. and Sonenshine, D. E. (eds.) 2005. Human Granulocytic Anaplasmosis (Ehrlichiosis) IN: Tick-Borne Diseases of Humans. pg. 218-238.

LabLink is published quarterly by the Michigan Department of Community Health, Bureau of Laboratories, to provide laboratory information to Michigan health professionals and the public health community.

Director, Bureau of Laboratories
Frances Pouch Downes, Dr.P.H.

Editor
Susan L. Shiflett